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(54) Title: ENZYMATIC DETERGENT COMPOSITION

(57) Abstract

A lipolytic enzyme with high activity at alkaline pH in the absence of Ca^{++} can be obtained from strains of filamentous fungi belonging to the genus Absidia. The lipolytic enzymes are effective for improving the effect of detergents towards fatty soiling.

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ENZYMATIC DETERGENT COMPOSITION

TECHNICAL FIELD

This invention relates to an enzymatic detergent composition and an enzymatic detergent additive comprising a lipolytic enzyme.

BACKGROUND ART

Lipolytic enzymes are known to be useful in detergents to improve the removal of fatty stains. Thus, in recent years Lipolase®, a microbial lipase derived from the fungus *Thermomyces lanuginosus* (also called *Humicola lanuginosa*), has been introduced into many commercial brands of detergent.

Other microbial lipases have also been suggested for use in detergents, e.g. bacterial lipase from *Pseudomonas cepacia* (US 4,876,024), from *Streptomycetes* (WO 94/14940) and from *Gongronella butleri* strain NRRL 3521 (US 3,634,195, the strain was previously named *Absidia butleri*, see K.H. Domsch et al., *Compendium of Soil Fungi*, Academic Press 1980, p. 381).

Many detergents are alkaline with a high pH in solution (e.g. around pH 10) and contain a builder to bind Ca^{++} ions, so there is a need for lipolytic enzymes with high activity at high pH in the absence of Ca^{++}.

SUMMARY OF THE INVENTION

Surprisingly, we have found that a lipolytic enzyme with high activity at alkaline pH in the absence of Ca^{++} can be obtained from strains of filamentous fungi belonging to the genus *Absidia* and that the lipolytic enzymes are effective for improving the effect of detergents.

Accordingly, the invention provides an enzymatic detergent composition comprising a surfactant and an alkaline *Absidia* lipolytic enzyme. The invention also provides a method for removing fatty soiling from textile, comprising washing the textile in an aqueous solution comprising the detergent composition.

The invention further provides an enzymatic detergent additive containing an *Absidia* lipolytic enzyme as an active component, provided in the form of a non-dusting granulate, a stabilized liquid, a slurry, or a protected enzyme.

Other aspects of the invention provide methods for producing an alkaline lipolytic enzyme derived from a lipolytic enzyme-producing strain of *Absidia reflexa* or *Absidia sporophora-variabilis*, either by cultivation of the strain or by recombinant DNA technology.

US 3,634,195 describes production of lipase from *A. cylindrospora var. rhizomorpha* NRRL 2815 and *A. blakesleeana* NRRL 1305. S. Koritala et al., *J.Am.Oil Chem.Soc.*, 64 (4), 509-13 (1987) discloses that soybean oil was partially hydrolyzed when incubated with *A. coerula* NRRL 5926 and *A. ramosa* NRRL 1309. *T. Satyanarayana, Current Science*, 50 (15), 680-2 (1981) discloses the secretion of lipase by a strain of *A. corymbifera*. K. Aisaka et al., *Agric. Biol. Chem.*, 43 (10), 2125-2129 (1979) describes the formation of a lipoprotein lipase from *Absidia hyalospora* strain KY 303 (now classified as *A. blakesleeana*).

However, the prior art does not disclose or suggest that lipolytic enzymes from *Absidia* are active at high pH in the absence of Ca^{++}, nor that they are useful in detergents.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-5 show graphs of lipolytic enzyme activity versus pH in the absence of Ca^{++} for some purified lipolytic enzymes according to the invention. Details are given in Example 8.

DETAILED DISCLOSURE OF THE INVENTION

Microorganisms

The microbial strain used in this invention belongs to the genus *Absidia*, as described in M.A.A. Schipper, *Persoonia*, Vol. 14, Part 2, pp. 133-148 (1990). Within this genus, the following subgenera, groups, species and strains are preferred. Variants and mutants thereof capable of producing lipolytic enzyme may also be used in the invention. It is noted that a number of previously recognized species names were reclassified by Schipper, *Op.cit.*, and for convenience the previously used names of some strains are also listed below.

The prior art does not describe lipolytic enzyme production from *A. reflexa* and *A. sporophora-variabilis*, two species which were not classified by

Schipper. The production of a lipolytic enzyme by these two species has not previously been described, and we have found that the lipolytic enzymes from these species are distinct from the lipolytic enzymes from the subgenera *Mycocladus* and *Absidia*.

Subgenus, group	Species name	Previous species name	Inventors' strain No.	Deposit number(s)
Subgenus *Mycocladus*	*A. blakesleeana*	*A. blakesleeana*	NN100826	NRRL 1304 ATCC 10148a CBS 100.28 CMI 111736
		A. blakesleeana	NN102406	CBS 100.36
		A. blakesleeana	NN102407	CBS 102.36 NRRL 2696
		A. blakesleeana	NN102408	CBS 420.70
		A. blakesleeana	NN102413	NRRL 1305
		A. griseola	NN000987	ATCC 20430
		A. griseola	NN102403	CBS 519.71 ATCC 22618 IFO 9472
		A. griseola var. iguchii	NN000591	ATCC 20431
		A. hyalospora	NN102432	CBS 173.67 NRRL 2916
	A. blakesleeana var. atrospora	*A. atrospora*	NN102423	CBS 518.71 ATCC 22617 IFO 9471
	A. corymbifera	*A. corymbifera*	NN100060	CBS 100.31 IFO 4009 NRRL 2982
		A. corymbifera	NN100062	IFO 8084

		A. corymbifera	NN102404	CBS 102.48
		A. corymbifera	NN102405	CBS 582.65 ATCC 22574 NRRL 1309
		A. hesseltinii	NN102426	CBS 958.68 ATCC 24263
Subgenus *Absidia*, Group B	*A. cylindrospora var. rhizomorpha*	-	NN102422	CBS 154.63 NRRL 2815
	A. pseudocylindrospora	-	NN102434	ATCC 24169 CBS 100.62 NRRL 2770
-	*A. reflexa*	-	NN102424	ATCC 44896 IFO 5874
-	*A. sporophora-variabilis*	-	NN102427	ATCC 36019

The above-mentioned strains are freely available from the following depositary institutions for microorganisms. Multiple numbers in the same box indicate multiple deposits of the same strain.

NRRL: Agricultural Research Service Culture Collection, 1815 North University Street, Peoria, Illinois 61604, USA.

ATCC: American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA.

CBS: Centraal Bureau voor Schimmelcultures, Oosterstraat 1, 3740 AG Baarn, Netherlands.

CMI: CAB International Mycological Institute, Ferry Lane, Kew, Surrey TW9 2AF, U.K.

IFO: Institute for Fermentation, 17-85 Juso-honmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.

Lipolytic enzyme may be produced by cultivating any of the above microorganisms in a suitable nutrient medium, optionally followed by recovery and

purification, according to methods well known in the art or as described in the examples of this specification.

Enzyme properties

The enzymes of this invention are lipolytic enzymes. In the present context the term "lipolytic enzyme" is intended to indicate an enzyme classified under the Enzyme Classification number E.C. 3.1.1.- (Carboxylic Ester Hydrolases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB). Lipolytic enzymes thus exhibit hydrolytic activity towards at least one of the types of ester bonds mentioned in the context of E.C. 3.1.1, e.g. ester bonds present in mono-, di- and triglycerides, phospholipids (all classes), thioesters, cholesterol esters, wax-esters, cutin, suberin, synthetic esters, etc. As an example, the lipolytic enzymes of the invention may have activity towards triglycerides (lipase activity, E.C. 3.1.1.3), e.g. 1,3-positionally specific lipase activity.

The lipolytic enzymes of this invention are characterized by having a high activity at alkaline pH (about pH 9-10), even in the absence of free Ca^{++}.

More specifically, these lipolytic enzymes have optimum activity at about pH 9 or higher (have a higher activity at pH 9 than at pH 8) when tested in the absence of free Ca^{++} by the OPID method described below.

Some preferred lipolytic enzymes have an activity of at least 3 OPID units/ml when tested at pH 9 without free Ca^{++} and a lipolytic enzyme concentration of 20 LU/ml (LU and OPID are lipolytic enzyme activity units defined below), i.e. a ratio between activities on olive oil and tributyrin of at least 0.15 OPID/LU. Such lipolytic enzymes can be derived from strains of *Absidia* subgenus *Mycocladus*, e.g. the species and strains listed above.

Another group of preferred lipolytic enzymes have a higher lipolytic enzyme activity at pH 10 than pH 9 in the absence of Ca^{++}. Such a lipolytic enzyme can be derived from *A. reflexa*, e.g. the strain listed above. This lipolytic enzyme is novel and is provided by the invention.

A further group of preferred lipolytic enzymes retains more than 90% residual activity after 30 minutes incubation at pH 10, 45°C. Such a lipolytic enzyme

can be derived from a strain of *A. sporophora-variabilis*, e.g. the strain listed above. This lipolytic enzyme is novel and is provided by the invention.

Lipase Activity Determination (LU)

One Lipase Unit (LU) is the amount of enzyme which liberates 1 μmol of titratable fatty acid per minute with tributyrin as substrate and gum arabic as emulsifier at 30.0°C, pH 7.0 (phosphate buffer).

Lipase Activity Determination (OPID)

The lipolytic enzyme activity without free Ca^{++} in the range pH 7-10 is tested with a substrate emulsion of olive oil: 2% PVA solution (1:3)at 40°C for 10 minutes, at a specified pH. At the end of the reaction, the reaction mixture is extracted by chloroform: methanol (1:1) at acidic conditions, and the fatty acid released during the reaction is measured by TLC-FID analysis (Iatroscan). One unit (OPIDU) is taken as the release of a μmole of fatty acid per minute.

In each test, 10 mM EDTA is used together with 200 mM of buffer (Tris-HCl buffer at pH 7 and 8, diethanol amine buffer at pH 8, 9 and 10).

Immunochemical Properties

Positionally non-specific lipolytic enzymes having immunochemical properties identical or partially identical to those of a lipolytic enzyme native to a strain of *Absidia* and having the stated properties are within the scope of the invention.

The immunochemical properties can be determined by immunological cross-reaction identity tests. The identity tests can be performed by the well-known Ouchterlony double immunodiffusion procedure or by tandem crossed immunoelectrophoresis according to I. M. Roitt; Immunology, Gower Medical Publishing (1985) and N. H. Axelsen; Handbook of Immunoprecipitation-in-Gel Techniques, Blackwell Scientific Publications (1983), Chapters 5 and 14. The terms immunochemical identity (antigenic identity) and partial immunochemical identity (partial antigenic identity) are described in Axelsen, *supra*, Chapters 5, 19 and 20 and Roitt, *supra*, Chapter 6.

Monospecific antiserum for use in immunological tests can be raised, e.g. in rabbits, against a purified lipolytic enzyme, e.g. as described in Chapter 41 of N.H. Axelsen, *supra* or Chapter 23 of N.H. Axelsen et al., A Manual of Quantitative Immunoelectrophoresis, Blackwell Scientific Publications (1973).

Production of lipolytic enzyme

The lipolytic enzyme of the invention may be produced by cultivation of one of the microorganisms described above in a suitable nutrient medium, containing carbon and nitrogen sources and inorganic salts, followed by recovery of the lipolytic enzyme.

After the cultivation, the lipolytic enzyme may be recovered and purified from the culture broth by conventional methods, such as hydrophobic chromatography, ion exchange chromatography and combinations thereof.

Convenient purification methods consist of an optional batch purification followed by two-step chromatography. The optional batch purification can be done by DEAE Streamline (product of Pharmacia), Super-Q Toyopearl, anion exchange resin or Macroprep HIC Support hydrophobic (product of Biorad). One part of the two-step chromatography may consist of hydrophobic chromatography, e.g. with Phenyl Toyopearl, Butyl Toyopearl or Macroprep HIC Support hydrophobic. The other part of the two-step chromatography may be done with an anion exchange resin, e.g. DEAE Toyopearl or Super-Q Toyopearl. The two steps may be carried in either sequence.

Application of lipolytic enzyme

The lipolytic enzyme of the invention may be used in conventional applications of lipolytic enzyme, particularly at a high pH, e.g. in laundry and dishwash detergents, in institutional and industrial cleaning and in leather processing.

The lipolytic enzymes of the invention can also be used for interesterification, for total hydrolysis of fats and oils and in optical isomer resolution processes.

Detergent additive

According to the invention, the lipolytic enzyme may typically be used as an additive in a detergent composition. This additive is conveniently formulated as a non-dusting granulate, a stabilized liquid, a slurry or a protected enzyme.

A suitable activity range for a detergent additive containing the lipolytic enzyme of this invention is 5,000-100,000 OPIDU/g (OPID measured at pH 9) or 0.01-100 mg pure enzyme protein per g of the additive.

Detergent

Advantageously, the lipolytic enzymes of this invention have high activity at alkaline pH (about pH 9-10), even in the absence of free Ca^{++}. This makes these lipolytic enzymes well suited for use in a wide range of detergents, even in detergents with a high content of builder to bind the free Ca^{++}.

The lipolytic enzyme of the invention may be incorporated in concentrations conventionally employed in detergents. The detergent composition of the invention may comprise lipolytic enzyme in an amount corresponding to 10-50,000 LU per gram of detergent, preferably 20-5,000 LU/g. The detergent may be dissolved in water to produce a wash liquor containing lipolytic enzyme in an amount corresponding to 25-15,000 LU per liter of wash liquor. The amount of lipolytic enzyme protein may be 0.001-10 mg per gram of detergent or 0.001-100 mg per liter of wash liquor.

Detergent composition

According to the invention, the lipolytic enzyme may typically be a component of a detergent composition. As such, it may be included in the detergent composition in the form of a non-dusting granulate, a stabilized liquid, or a protected enzyme. Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethylene glycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms

and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in patent GB 1483591. Liquid enzyme preparations may, for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

The detergent composition of the invention may be in any convenient form, e.g. as powder, granules, paste or liquid. A liquid detergent may be aqueous, typically containing up to 70% water and 0-30% organic solvent, or nonaqueous.

The detergent composition comprises one or more surfactants, each of which may be anionic, nonionic, cationic, or zwitterionic. The detergent will usually contain 0-50% of anionic surfactant such as linear alkylbenzene sulfonate (LAS), alpha-olefin sulfonate (AOS), alkyl sulfate (fatty alcohol sulfate) (AS), alcohol ethoxysulfate (AEOS or AES), secondary alkane sulfonates (SAS), alpha-sulfo fatty acid methyl esters, alkyl- or alkenylsuccinic acid, or soap. It may also contain 0-40% of nonionic surfactant such as alcohol ethoxylate (AEO or AE), carboxylated alcohol ethoxylates, nonylphenol ethoxylate, alkylpolyglycoside, alkyldimethylamine oxide, ethoxylated fatty acid monoethanolamide, fatty acid monoethanolamide, or polyhydroxy alkyl fatty acid amide (e.g. as described in WO 92/06154).

The detergent composition may additionally comprise one or more other enzymes, such as amylase, cutinase, protease, cellulase, peroxidase, and oxidase, e.g., laccase.

The detergent may contain 1-65% of a detergent builder or complexing agent such as zeolite, diphosphate, triphosphate, phosphonate, citrate, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTMPA), alkyl- or alkenylsuccinic acid, soluble silicates or layered silicates (e.g. SKS-6 from Hoechst). The detergent may also be unbuilt, i.e. essentially free of detergent builder.

The detergent may comprise one or more polymers. Examples are carboxymethyl cellulose (CMC), poly(vinyl pyrrolidone) (PVP), polyethylene glycol

(PEG), poly(vinyl alcohol) (PVA), polycarboxylates such as polyacrylates, maleic/acrylic acid copolymers and lauryl methacrylate/acrylic acid copolymers.

The detergent may contain a bleaching system which may comprise a H_2O_2 source such as perborate or percarbonate which may be combined with a peracid-forming bleach activator such as tetraacetylethylenediamine (TAED) or nonanoyloxybenzene sulfonate (NOBS). Alternatively, the bleaching system may comprise peroxy acids of, e.g., the amide, imide, or sulfone type.

The enzymes of the detergent composition of the invention may be stabilized using conventional stabilizing agents, e.g. a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative such as, e.g., an aromatic borate ester, and the composition may be formulated as described in, e.g., WO 92/19709 and WO 92/19708.

The detergent may also contain other conventional detergent ingredients such as, e.g., fabric conditioners including clays, foam boosters, suds suppressors, anti-corrosion agents, soil-suspending agents, anti-soil-redeposition agents, dyes, bactericides, optical brighteners, or perfume.

The pH (measured in aqueous solution at use concentration) will usually be neutral or alkaline, e.g. in the range of 7-11.

Particular forms of detergent compositions within the scope of the invention include:

1) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzene sulfonate (calculated as acid)	7 - 12%
Alcohol ethoxysulfate (e.g. C_{12-18} alcohol, 1-2 EO) or alkyl sulfate (e.g. C_{16-18})	1 - 4%
Alcohol ethoxylate (e.g. C_{14-15} alcohol, 7 EO)	5 - 9%
Sodium carbonate (as Na_2CO_3)	14 - 20%
Soluble silicate (as $Na_2O,2SiO_2$)	2 - 6%
Zeolite (as $NaAlSiO_4$)	15 - 22%
Sodium sulfate (as Na_2SO_4)	0 - 6%

Linear alkylbenzene sulfonate (calculated as acid)	7 - 12%
Sodium citrate/citric acid (as $C_6H_5Na_3O_7/C_6H_8O_7$)	0 - 15%
Sodium perborate (as $NaBO_3.H_2O$)	11 - 18%
TAED	2 - 6%
Carboxymethyl cellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	0 - 3%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume, optical brightener, photobleach)	0 - 5%

2) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzene sulfonate (calculated as acid)	6 - 11%
Alcohol ethoxysulfate (e.g. C_{12-18} alcohol, 1-2 EO or alkyl sulfate (e.g. C_{16-18})	1 - 3%
Alcohol ethoxylate (e.g. C_{14-15} alcohol, 7 EO)	5 - 9%
Sodium carbonate (as Na_2CO_3)	15 - 21%
Soluble silicate (as $Na_2O,2SiO_2$)	1 - 4%
Zeolite (as $NaAlSiO_4$)	24 - 34%
Sodium sulfate (as Na_2SO_4)	4 - 10%
Sodium citrate/citric acid (as $C_6H_5Na_3O_7/C_6H_8O_7$)	0 - 15%
Carboxymethyl cellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	1 - 6%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume)	0 - 5%

3) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzene sulfonate (calculated as acid)	5 - 9%
Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO)	7 - 14%

Linear alkylbenzene sulfonate (calculated as acid)	5 - 9%
Soap as fatty acid (e.g. C_{16-22} fatty acid)	1 - 3%
Sodium carbonate (as Na_2CO_3)	10 - 17%
Soluble silicate (as $Na_2O,2SiO_2$)	3 - 9%
Zeolite (as $NaAlSiO_4$)	23 - 33%
Sodium sulfate (as Na_2SO4)	0 - 4%
Sodium perborate (as $NaBO_3.H_2O$)	8 - 16%
TAED	2 - 8%
Phosphonate (e.g. EDTMPA)	0 - 1%
Carboxymethyl cellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	0 - 3%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume, optical brightener)	0 - 5%

4) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Linear alkylbenzene sulfonate (calculated as acid)	8 - 12%
Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO)	10 - 25%
Sodium carbonate (as Na_2CO_3)	14 - 22%
Soluble silicate (as $Na_2O,2SiO_2$)	1 - 5%
Zeolite (as $NaAlSiO_4$)	25 - 35%
Sodium sulfate (as Na_2SO_4)	0 - 10%
Carboxymethyl cellulose	0 - 2%
Polymers (e.g. maleic/acrylic acid copolymer, PVP, PEG)	1 - 3%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume)	0 - 5%

5) An aqueous liquid detergent composition comprising

Linear alkylbenzene sulfonate (calculated as acid)	15 - 21%

Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO or C_{12-15} alcohol, 5 EO)	12 - 18%
Soap as fatty acid (e.g. oleic acid)	3 - 13%
Alkenylsuccinic acid (C_{12-14})	0 - 13%
Aminoethanol	8 - 18%
Citric acid	2 - 8%
Phosphonate	0 - 3%
Polymers (e.g. PVP, PEG)	0 - 3%
Borate (as B_4O_7)	0 - 2%
Ethanol	0 - 3%
Propylene glycol	8 - 14%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. dispersants, suds suppressors, perfume, optical brightener)	0 - 5%

6) An aqueous structured liquid detergent composition comprising

Linear alkylbenzene sulfonate (calculated as acid)	15 - 21%
Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO, or C_{12-15} alcohol, 5 EO)	3 - 9%
Soap as fatty acid (e.g. oleic acid)	3 - 10%
Zeolite (as $NaAlSiO_4$)	14 - 22%
Potassium citrate	9 - 18%
Borate (as B_4O_7)	0 - 2%
Carboxymethyl cellulose	0 - 2%
Polymers (e.g. PEG, PVP)	0 - 3%
Anchoring polymers such as, e.g., lauryl methacrylate/acrylic acid copolymer; molar ratio 25:1; MW 3800	0 - 3%
Glycerol	0 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%

Linear alkylbenzene sulfonate (calculated as acid)	15 - 21%
Minor ingredients (e.g. dispersants, suds suppressors, perfume, optical brighteners)	0 - 5%

7) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Fatty alcohol sulfate	5 - 10%
Ethoxylated fatty acid monoethanolamide	3 - 9%
Soap as fatty acid	0 - 3%
Sodium carbonate (as Na_2CO_3)	5 - 10%
Soluble silicate (as $Na_2O,2SiO_2$)	1 - 4%
Zeolite (as $NaAlSiO_4$)	20 - 40%
Sodium sulfate (as Na_2SO_4)	2 - 8%
Sodium perborate (as $NaBO_3.H_2O$)	12 - 18%
TAED	2 - 7%
Polymers (e.g. maleic/acrylic acid copolymer, PEG)	1 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. optical brightener, suds suppressors, perfume)	0 - 5%

8) A detergent composition formulated as a granulate comprising

Linear alkylbenzene sulfonate (calculated as acid)	8 - 14%
Ethoxylated fatty acid monoethanolamide	5 - 11%
Soap as fatty acid	0 - 3%
Sodium carbonate (as Na_2CO_3)	4 - 10%
Soluble silicate (as $Na_2O,2SiO_2$)	1 - 4%
Zeolite (as $NaAlSiO_4$)	30 - 50%
Sodium sulfate (as Na_2SO_4)	3 - 11%
Sodium citrate (as $C_6H_5Na_3O_7$)	5 - 12%
Polymers (e.g. PVP, maleic/acrylic acid copolymer, PEG)	1 - 5%

Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. suds suppressors, perfume)	0 - 5%

9) A detergent composition formulated as a granulate comprising

Linear alkylbenzene sulfonate (calculated as acid)	6 - 12%
Nonionic surfactant	1 - 4%
Soap as fatty acid	2 - 6%
Sodium carbonate (as Na_2CO_3)	14 - 22%
Zeolite (as $NaAlSiO_4$)	18 - 32%
Sodium sulfate (as Na_2SO_4)	5 - 20%
Sodium citrate (as $C_6H_5Na_3O_7$)	3 - 8%
Sodium perborate (as $NaBO_3.H_2O$)	4 - 9%
Bleach activator (e.g. NOBS or TAED)	1 - 5%
Carboxymethyl cellulose	0 - 2%
Polymers (e.g. polycarboxylate or PEG)	1 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. optical brightener, perfume)	0 - 5%

10) An aqueous liquid detergent composition comprising

Linear alkylbenzene sulfonate (calculated as acid)	15 - 23%
Alcohol ethoxysulfate (e.g. C_{12-15} alcohol, 2-3 EO)	8 - 15%
Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO, or C_{12-15} alcohol, 5 EO)	3 - 9%
Soap as fatty acid (e.g. lauric acid)	0 - 3%
Aminoethanol	1 - 5%
Sodium citrate	5 - 10%
Hydrotrope (e.g. sodium toluene sulfonate)	2 - 6%
Borate (as B_4O_7)	0 - 2%
Carboxymethyl cellulose	0 - 1%

Ethanol	1 - 3%
Propylene glycol	2 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. polymers, dispersants, perfume, optical brighteners)	0 - 5%

11) An aqueous liquid detergent composition comprising

Linear alkylbenzene sulfonate (calculated as acid)	20 - 32%
Alcohol ethoxylate (e.g. C_{12-15} alcohol, 7 EO, or C_{12-15} alcohol, 5 EO)	6 - 12%
Aminoethanol	2 - 6%
Citric acid	8 - 14%
Borate (as B_4O_7)	1 - 3%
Polymer (e.g. maleic/acrylic acid copolymer, anchoring polymer such as, e.g., lauryl methacrylate/acrylic acid copolymer)	0 - 3%
Glycerol	3 - 8%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. hydrotropes, dispersants, perfume, optical brighteners)	0 - 5%

12) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

Anionic surfactant (linear alkylbenzene sulfonate, alkyl sulfate, alpha-olefin sulfonate, alpha-sulfo fatty acid methyl esters, alkane sulfonates, soap)	25 - 40%
Nonionic surfactant (e.g. alcohol ethoxylate)	1 - 10%
Sodium carbonate (as Na_2CO_3)	8 - 25%
Soluble silicates (as Na_2O, $2SiO_2$)	5 - 15%
Sodium sulfate (as Na_2SO_4)	0 - 5%
Zeolite (as $NaAlSiO_4$)	15 - 28%
Sodium perborate (as $NaBO_3.4H_2O$)	0 - 20%

Bleach activator (TAED or NOBS)	0 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. perfume, optical brighteners)	0 - 3%

13) Detergent formulations as described in 1) - 12) wherein all or part of the linear alkylbenzene sulfonate is replaced by (C_{12}-C_{18}) alkyl sulfate.

14) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

(C_{12}-C_{18}) alkyl sulfate	9 - 15%
Alcohol ethoxylate	3 - 6%
Polyhydroxy alkyl fatty acid amide	1 - 5%
Zeolite (as $NaAlSiO_4$)	10 - 20%
Layered disilicate (e.g. SK56 from Hoechst)	10 - 20%
Sodium carbonate (as Na_2CO_3)	3 - 12%
Soluble silicate (as $Na_2O,2SiO_2$)	0 - 6%
Sodium citrate	4 - 8%
Sodium percarbonate	13 - 22%
TAED	3 - 8%
Polymers (e.g. polycarboxylates and PVP=	0 - 5%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. optical brightener, photo bleach, perfume, suds suppressors)	0 - 5%

15) A detergent composition formulated as a granulate having a bulk density of at least 600 g/l comprising

(C_{12}-C_{18}) alkyl sulfate	4 - 8%
Alcohol ethoxylate	11 - 15%
Soap	1 - 4%

Zeolite MAP or zeolite A	35 - 45%
Sodium carbonate (as Na_2CO_3)	2 - 8%
Soluble silicate (as $Na_2O,2SiO_2$)	0 - 4%
Sodium percarbonate	13 - 22%
TAED	1 - 8%
Carboxymethyl cellulose	0 - 3%
Polymers (e.g. polycarboxylates and PVP)	0 - 3%
Enzymes (calculated as pure enzyme protein)	0.0001 - 0.1%
Minor ingredients (e.g. optical brightener, phosphonate, perfume)	0 - 3%

16) Detergent formulations as described in 1) - 15) which contain a stabilized or encapsulated peracid, either as an additional component or as a substitute for already specified bleach systems.

17) Detergent compositions as described in 1), 3), 7), 9) and 12) wherein perborate is replaced by percarbonate.

18) Detergent compositions as described in 1), 3), 7), 9), 12), 14) and 15) which additionally contain a manganese catalyst. The manganese catalyst may, e.g., be one of the compounds described in "Efficient manganese catalysts for low-temperature bleaching", Nature 369, 1994, pp. 637-639.

19) Detergent composition formulated as a nonaqueous detergent liquid comprising a liquid nonionic surfactant such as, e.g., linear alkoxylated primary alcohol, a builder system (e.g. phosphate), enzyme and alkali. The detergent may also comprise anionic surfactant and/or a bleach system.

EXAMPLES

Culture media

The culture media shown in the table below were used in the examples.

Ingredient	Composition of medium (g/L)											
	MR-10	MT-O	OM	OMM	RS-G	YS-2	YS-2SO	ToMa1	ToMa5	MT-O	ToMa10	YPG
Pharmamedia	10				20				30		15	
Soybean powder	10	30			40			30		30		
Yeast extract		1			-	10	10			1		4
Peptone		5			-	10	10			5		
Corn steep powder		5								5		
Glucose		10			-	20	20			10		15
Sucrose	2										2.5	
Glycerol								10				
Dried yeast			30	30								
Oatmeal agar (Difco)				3								
Corn steep liquor					10							
Urea					5[a]			1			0.5	
Oatmeal agar (ISP No. 3 Difco)			3									
KH_2PO_4	5	4			1		5	5	5	4	2.5	1
$MgSO_4 \cdot 7H_2O$	0.2	0.2	0.2	0.5	0.5	1	1	1	0.4	0.1	0.5	0.5

Ingredient	Composition of medium (g/L)											
	MR-10	MT-O	OM	OMM	RS-G	YS-2	YS-2SO	ToMa1	ToMa5	MT-O	ToMa10	YPG
NH_4NO_3		2.5								2.5		
$(NH_4)_2SO_4$								1.5				
Olive oil, ml/100 ml	2	2	1		2					2		
Soybean oil, ml/100 ml							2					
Jojoba oil, ml/100 ml									1			
Soy lecithin, ml/100 ml									2			
Methyl oleate, ml/100 ml								2				
Sorbitan ester (Tween 40), ml/100 ml											3	
$CaCO_3$, tablets/100 ml					2							
pH adjusted to	6.5	-	6.2	6.2	5.5	6.5	6.5	7.5	7.5	7	7.5	6.0

x filtered separately

20

EXAMPLE 1

Production of lipolytic enzyme from *A. corymbifera*

A. corymbifera strain NN100062 was cultivated for 3 days at 30°C in shake flasks containing 100 ml of RS-G medium. 2,500 ml of cell-free broth was recovered from 50 shake flasks after removal of cell mass. This was freeze dried to obtain 58 g of powder sample with a lipolytic enzyme activity of 379 LU/g which was used in the following example.

EXAMPLE 2

Washing effect of *A. corymbifera* lipolytic enzyme

The washing effect of lipolytic enzyme (powder preparation from the previous example) was evaluated by washing of soiled textile in detergent containing anionic surfactant (LAS) at pH 10. The test was done in a Terg-O-tometer laboratory washing machine at the following conditions:

Temperature	30°C
Time	30 minutes
Agitation	100 rpm
Detergent	0.25 g/l of LAS (linear alkylbenzene sulfonate, product name Nansa HS 80/S) + 1.0 g/l of Na_2CO_3
Water	Tap water (approx. 18° German hardness)
pH	10.0
Lipolytic enzyme dosage	2,000 LU/l
Test material	Cotton cloth, 7 x 7 cm, each stained with 85 µL of olive oil
Cloth/liquid ratio	7 swatches/500 ml

After washing, the swatches were Soxhlet extracted, and the residual amount of oil was determined gravimetrically. The composition of the residual oil was determined by TLC/FID analyses. A control experiment without lipolytic enzyme was made in the same manner. Results:

	Without lipolytic enzyme	With lipolytic enzyme
Residual oil (mg)	396	338
Composition of residual oil:		
% triglyceride	92	32
% free fatty acid	4	53
% 1,3-diglyceride	2	9
% 1,2-diglyceride	2	5
% monoglyceride	0	0

It is seen that the lipolytic enzyme is effective in reducing the total amount residual oil and particularly reducing the amount of triglyceride in washing at pH 10.0 in the presence of LAS.

EXAMPLE 3

Production and purification of lipolytic enzyme from *A. blakesleeana*

In this example, lipolytic enzyme activity was determined using olive oil emulsified with gum arabic. Conditions were 40°C, pH 10 (100 mM glycine buffer). 1 unit was taken as the amount of enzyme which liberates a titratable amount of fatty acid equivalent to 1 μmole of NaOH per minute.

A. blakesleeana strain NN100826 was cultivated for 3 days at 30°C in shake flasks containing 100 ml of OM medium. The lipolytic enzyme yield was 41 units/ml.

Culture broth was collected from 50 shake flasks and concentrated to 2 L by ultrafiltration after washing with 2 L of deionized water. Ground ammonium sulfate was added to the concentrated broth under stirring in a cold chamber up to 40% saturation and left for 1 hour at 4°C. The precipitate was removed by centrifugation. Ammonium sulfate was further added to 50% saturation and the precipitate was removed. The supernatant was concentrated to 180 ml and dialyzed

overnight using cellulose tube in 20 mM Tris/HCl buffer (pH 8.5) at 4°C and freeze dried. 9.8 g of powder lipolytic enzyme preparation was obtained, having an activity of 1500 units/g.

Another powder lipolytic enzyme preparation was obtained by addition of chilled acetone to culture broth and freeze-drying of the precipitate.

EXAMPLE 4

Washing effect of *A. blakesleeana* lipolytic enzyme

Powder lipolytic enzyme preparation from the previous example was tested in the same manner as the previous washing example with the following changes: The washing time was 20 minutes; each swatch of cloth was stained with 50 µL of oil; the swatches were aged for 2 days at room temperature before the washing test; and the pH, detergents and lipolytic enzyme dosages were as shown below. The analysis data were used to calculate the residual ester bonds (in µmoles) and the degree of hydrolysis.

Detergent	pH	Lipolytic enzyme dosage (LU/l)	Residual oil (mg)	Residual ester bonds (µmoles)	DH (%)
0.25 g/l LAS + 0.25 g/l AE + 1.0 g/l Na_2CO_3	9.5	0	185	607	3.2
		800	166	466	16.0
		2500	138	339	26.3
0.5 g/l LAS + Na_2CO_3	9	0	171	556	4.1
		2500	158	496	7.1
	10	0	171	559	3.6
		2500	153	462	10.4
	11	0	168	544	4.5
		2500	162	509	6.7

It is seen that the lipolytic enzyme is effective in reducing the amount of residual oil and increasing the degree of hydrolysis, thus lowering the number of residual ester bonds.

EXAMPLE 5

Production of lipolytic enzyme from *A. blakesleeana*

A. blakesleeana strain NN100826 was allowed to sporulate for 5 weeks on a slant of 39 g/l of PDA (product of Difco) and 10 g/l of agar in water.

9 ml of a 0.1% solution of Tween in water was poured onto the slant to make a spore suspension.

3 ml of the spore suspension was inoculated into a 500 ml baffled shake flask (two baffles) containing 100 ml of YS-2SO medium, and the flask was incubated with shaking (230 rpm) at 34°C for 24 hours to prepare a seed culture.

The seed culture was homogenized to break up a pellet-shaped mycelium, and 2 ml of the homogenized culture was inoculated into a shake flask containing 100 ml of OMM medium and 2% of soybean lecithin. The flask was incubated with shaking (230 rpm) at 30°C. After 2 days cultivation, the broth had a lipolytic enzyme activity of 17.0 LU/ml and a pH of 6.7.

EXAMPLE 6

Purification of lipolytic enzyme

Lipolytic enzyme from *A. blakesleeana* strain NN100826 was purified by three step chromatography, namely Streamline DEAE, Phenyl- and DEAE-Toyopearl, as follows.

Streamline DEAE column chromatography. Culture broth from 50 shake flasks prepared as in the previous example was centrifuged to obtain 2.8 L of a cell-free broth. This was applied onto 600 ml of Streamline DEAE pre-equilibrated with 50 mM sodium carbonate buffer, pH 10. Flow rate was 100 ml/min. After washing the column with the same buffer, bound lipolytic enzyme was eluted by 50 mM Tris buffer containing 0.6 M NaCl, pH 7.2. 38% and 36% of the starting lipolytic enzyme activity was recovered in the eluate and the pass-through fraction, respectively, i.e. a total recovery of 74%. For further purification the lipolytic enzyme bound to resin

was used. The lipolytic enzyme solution was neutralized, then concentrated by UF module, 3000 NMWL. Recovery was 47%. After concentration the lipolytic enzyme was filtered through 0.2 μm membrane.

Phenyl Toyopearl column chromatography. It had been found that with gradient elution the lipolytic enzyme activity gave a very broad peak which was difficult to detect. Instead, step elution was used with 60 minutes of 1.4 M ammonium acetate, followed by 30 minutes of pure water and 30 minutes of 20% ethanol. The lipolytic enzyme activity gave two peaks. One was eluted by water ("lipolytic enzyme A") and the other eluted by 20% EtOH ("lipolytic enzyme B"). Recovery was 41% for lipolytic enzyme A and 33% for lipolytic enzyme B. Each lipolytic enzyme was concentrated and deionized by UF module, 3,000 NMWL. Recovery was 94% and 91%, respectively.

DEAE Toyopearl column chromatography. Lipolytic enzyme A was purified by gradient elution from 50 mM sodium carbonate buffer (pH 10) to 50 mM Tris buffer (pH 7.2) + 0.6 M NaCl. Fractions with high lipolytic enzyme activity were pooled. The yield was 66%. The lipolytic enzyme was concentrated and deionized by UF module, 3,000 NMWL. Recovery was 69%.

SDS-PAGE showed the lipolytic enzyme to be pure with a single protein band. It was found to have isoelectric point at pH 8.0 and molecular weight 25,400. The specific activity of the pure lipolytic enzyme was found to be 3,300 - 4,100 LU/mg.

Lipolytic enzyme B was purified in a similar manner, and it was confirmed by SDS-PAGE that it was identical to lipolytic enzyme A.

EXAMPLE 7

Production of lipolytic enzyme from various *Absidia* strains

Each of the *Absidia* strains shown in the table below was used for lipolytic enzyme production by the following steps.

Seed culture. 2 days at 27°C on YS-2 medium (omitted for NN100826).

Main culture. In shake flasks using the indicated medium at 27°C (30°C in one case, as noted). The cultivation time and lipolytic enzyme yield obtained are also shown in Table 2.

Recovery and purification. Centrifugation to get cell-free samples, followed by freeze-drying to make powder samples.

The culture conditions and the resulting yields are given below

Species	Strain No.	Seed medium	Main medium	Days	Yield LU/ml
A. blakesleeana	NN000591	YS-2	MR-10	4	8.3
A. blakesleeana	NN000987	YS-2	MT-O	4	4.5
A. blakesleeana	NN100826	None	OMM + 2% lecithin	2 (30°C)	17.0
A. blakesleeana	NN102403	YS-2	MT-O	4	3.0
A. blakesleeana	NN102406	YS-2	OM	4	3.2
A. blakesleeana	NN102407	YS-2	MT-O	4	2.6
A. blakesleeana	NN102408	YS-2	MT-O	4	4.9
A. blakesleeana	NN102413	YS-2	MR-10	3	1.1
A. corymbifera	NN100062	YS-2	MT-O	5	32.0
A. corymbifera	NN102404	YS-2	MT-O	4	7.0
A. corymbifera	NN102405	YS-2	MR-10	4	6.9
A. corymbifera	NN100060	YPG	ToMa1	5	45
A. reflexa	NN102424	YPG	ToMa1	5	16
A. blakesleeana	NN102407	YS-2	ToMa5	5	40
A. blakesleeana	NN102408	YS-2	ToMa5	6	25
A. blakesleeana	NN000987	YPG	ToMa1	5	30

Species	Strain No.	Seed medium	Main medium	Days	Yield LU/ml
A. blakesleeana	NN102413	YPG	ToMa1	6	20
A. blakesleeana var. atrospora	NN102423	YS-2	ToMa5	6	20
A. corymbifera	NN102426	YPG	ToMa1	5	22
A. sporophora-variabilis	NN102427	YS-2	ToMa1	4	20
A. blakesleeana	NN102432	YS-2	ToMa5	5	15
A. blakesleeana	NN100826	YS-2	ToMa5	5	40
A. corymbifera	NN100062	YPG	ToMa1	5	70
A. blakesleeana	NN000591	YPG	ToMa1	5	70
A. blakesleeana	NN102403	YPG	ToMa1	5	40
A. corymbifera	NN102404	YPG	ToMa1	4	30
A. corymbifera	NN102405	YS-2	ToMa1	5	30
A. blakesleeana	NN102406	YS-2	ToMa5	6	30
A. cylindrospora var. rhizomorpha	NN102422	YPG	ToMa10	5	3.2 (pH 9)
A. pseudo-cylindrospora	NN102434	YS-2	MT-O	5	0-1

EXAMPLE 8

Effect of pH and Ca^{++} on activity of *Absidia* lipolytic enzymes

The lipolytic enzyme activity was tested in the range pH 7-10 without Ca^{++} by the OPID method described above, using a lipolytic enzyme amount of 20 LU/ml. Purified lipolytic enzymes according to the invention from the following strains were tested:

A. blakesleeana NN000591

A. blakesleeana NN000987

A. blakesleeana NN100826

A. corymbifera NN100062

A. reflexa NN102424

The results are shown in the enclosed figures.

It is seen that in the absence of Ca^{++}, all the *Absidia* lipolytic enzymes tested show higher activity at pH 9 than pH 8 (optimum at about pH 9 or higher), and the lipolytic enzyme from *A. reflexa* shows higher activity at pH 10 than pH 9 (optimum at about pH 10 or higher). It is also seen that the lipolytic enzymes from *Absidia* subgenus *Mycocladus* (represented by *A. blakesleeana* and *A. corymbifera*) show an activity at pH 9 in the absence of Ca^{++} above 3 OPIDU/ml for a lipolytic enzyme dosage of 20 LU/ml, i.e. a ratio of above 0.15 OPIDU/LU.

EXAMPLE 9

Plate test for lipolytic enzyme activity at pH 10

The plate test described in Example 11 of WO 88/02775 (corresponding to JP-W 1-501120) was used to check for lipolytic enzyme activity at pH 10 with and without the addition of Ca^{++}. Lipolytic enzyme preparations from all the strains listed in Example 7 were found to exhibit lipolytic enzyme activity at pH 10, both with and without Ca^{++} addition:

EXAMPLE 10

pI and MW of lipolytic enzymes

Purified lipolytic enzymes from some strains were used to determine the iso-electric point (pI) by preparative iso-electric focusing and the molecular weight (MW) by SDS-PAGE. Results:

Species	Strain No.	pI	MW
A. blakesleeana	NN100826	8	25 kDa
A. corymbifera	NN100062	5.2-5.8	32 kDa (SDS)
A. blakesleeana	NN000987	6.5	30 kDa
A. blakesleeana	NN000591	6.5	30 kDa
A. reflexa	NN102424	4.1	-
A. sporophoro-variabilis	NN102427	3.6-5	-

A separate purification of the lipase from *A. blakesleeana* NN100826 suggests the size to be 31-32 KDa. The 25 KDa lipase therefore probably represents a slightly truncated lipase molecule.

EXAMPLE 11

Structural characterization of *A. blakesleeana* lipolytic enzymes

The N-terminal sequences of lipolytic enzymes from *A. blakesleeana* NN000591 and NN000987 were determined following electroblotting. Both lipolytic enzymes have a molecular weight of around 30 kDa.

The N-terminal acid sequencing of the lipolytic enzyme from NN000987 gave the sequence shown as SEQ ID NO: 1 in the enclosed sequence listing.

The N-terminal sequencing of the lipolytic enzyme from NN000591 gave two sequences shown as SEQ ID NO: 2 and SEQ ID NO: 3.

It is seen that for NN000591, the N-terminal sequence shown as SEQ ID NO: 3 starts at amino acid residue 6 of the N-terminal sequence shown as SEQ ID NO: 2. Thus, the two sequences represent variable processing of the same protein either during synthesis or purification. In addition, it is clear that SEQ ID NO: 1 for NN000987 and SEQ ID NO: 2 for NN000591 represent the same N-terminal sequence, and it is believed that the two lipolytic enzymes are most likely identical.

Thus, based on the 3 above N-terminal sequences, it is concluded that the mature lipolytic enzyme has the N-terminal sequence shown as SEQ ID NO: 4.

In addition to the 30 kDa lipolytic enzyme in the NN000591 preparation, a band with molecular weight around 21 kDa was seen. N-terminal amino acid sequencing of this protein following electroblotting gave the sequence shown as SEQ ID NO: 5. This N-terminal sequence could be aligned to the lid of the known sequence for the lipase from *Rhizomucor miehei*, so it was concluded that it is a fragment of the full-length 30 kDa lipolytic enzyme.

The NN000591 lipolytic enzyme was reduced and S-carboxymethylated before degradation with a lysyl-specific protease. The resulting peptides were fractionated and re-purified using reversed phase HPLC before being subjected to N-terminal amino acid sequencing. The peptide sequences shown as SEQ ID NO: 6-10 were obtained.

By aligning the sequences with the known sequences of the lipases from *Rhizomucor miehei* and *Rhizopus delemar*, it was concluded that the full-length lipolytic enzyme contains the sequences SEQ ID NO: 4-10 in this order. In these sequences, Xaa represents an amino acid that could not be identified. Asx designates positions where Asp and Asn could not be distinguished. The amino acids in positions 1 and 9 of SEQ ID NO: 5 are uncertain.

EXAMPLE 12

Purification of *A. corymbifera* lipolytic enzyme

Lipolytic enzyme from *A. corymbifera* strain NN100062 was purified as follows.

Streamline. Crude lipolytic enzyme powder obtained by cultivation of the strain was dissolved in 50 mM sodium carbonate buffer (pH 10). After centrifugation, lipolytic enzyme sample was adsorbed on expanded DEAE resin equilibrated with the same buffer, and then the resin was washed with the same buffer. The lipolytic enzyme was eluted with Tris-HCl buffer (pH 7.6) containing 0.5 M NaCl. The yield of this step was 52%.

Butyl Toyopearl

The second step was hydrophobic column chromatography using pre-packed Butyl Toyopearl and HPLC. The concentrated lipolytic enzyme was adjusted to a salt concentration of 1 M ammonium acetate and then adsorbed on a column equilibrated with 1 M ammonium acetate. Elution was carried out with a linear gradient of 1-0 M ammonium acetate and 20% ethanol. The lipolytic enzyme activity of each fraction was measured, and the fractions with high lipolytic enzyme activity were gathered and desalted with micro asilizer (product of Asahi Kasei).

DEAE Toyopearl column chromatography. The third step was anion column chromatography using pre-packed DEAE Toyopearl and HPLC (product of Waters). The lipolytic enzyme was adjusted to pH 8.5. This was applied to a column equilibrated with 50 mM Tris-HCl buffer (pH 8.5), and the lipolytic enzyme was eluted with a linear gradient of 0-0.5 M NaCl. The fractions with high lipolytic enzyme activity were gathered, and the obtained lipolytic enzyme was concentrated. The yield of this step was 66%.

Gel filtration. The final step was gel filtration. The buffer used was 50 mM Tris-HCl containing 0.15 M NaCl. Again, the fractions with high lipolytic enzyme activity were gathered.

The purification is summarized in the following table.

Step	Activity (LU)	Specific activity (LU/mg)	Yield (%)
Powder	135500	18	100
STREAM LINE	69840	18	52
Butyl Toyopearl	28210	215	21
DEAE Toyopearl	15500	4250	8.5
Gel filtration	10140	5200	7.5

EXAMPLE 13

Structural characterization of *A. corymbifera* lipolytic enzyme

The structure of the lipolytic enzyme of *A. corymbifera* NN100062 was studied in the same manner as in Example 11. The N-terminal sequencing gave the sequence shown as SEQ ID NO: 11. Peptides obtained after degradation were found to have the sequences shown as SEQ ID NO: 12-16 and 18-19. It was found that the residue Asn20 of SEQ ID NO: 12 was glycosylated.

A comparison showed that 22 amino acids at the C-terminal of SEQ ID NO: 15 are identical to those at the N-terminal of SEQ ID NO: 16, and it was concluded that these two sequences form part of a larger fragment shown as SEQ ID NO: 17. By alignment with the known sequences of the lipases from *Rhizomucor miehei* and *Rhizopus delemar*, it was concluded that the full-length lipolytic enzyme contains the sequences SEQ ID NO: 11-14 and 17-19 in this order.

EXAMPLE 14

Alkaline stability of lipolytic enzyme from *A. sporophora-variabilis*

An enzyme solution was prepared containing approx. 10 LU/ml of lipolytic enzyme from *A. sporophora-variabilis* NN102427 in 50 mM glycine buffer at pH 10. A portion of this solution was incubated for 30 minutes at 45°C and rapidly cooled. The lipolytic enzyme activity was determined before and after the incubation.

The results showed that 97% residual activity remained after the incubation.

EXAMPLE 15

Substrate affinity of lipolytic enzyme from *A. sporophora-variabilis*

The following procedure was used for a simple determination of the ability of a lipolytic enzyme to accumulate on/in a substrate phase (olive oil) at alkaline pH (pH 9.0) in the presence of non-ionic surfactant Dobanol 25-7 (2500 ppm).

Two identical solutions of the lipolytic enzyme in buffer with non-ionic surfactant were prepared in sealable vials, and substrate was added to one of the solutions. Both solutions were incubated with vigorous shaking, and the remaining

lipolytic enzyme activity was determined (in LU, defined above) after separation and removal of the substrate.

The following conditions were used:

Buffer:	100 mM Glycine (pH 9.0)
Non-ionic surfactant	100 ppm alcohol ethoxylate (Dobanol™ 25-7)
Substrate:	Olive oil
Buffer : substrate	50:50 v/v
Incubation temperature	4°C.
Initial lipolytic enzyme activity	5-10 LU/ml
Incubation time	Over night (24-26 hours).

Lipolase® (a commercially available fungal lipolytic enzyme) was used for comparison. The results are given as the residual activity after incubation with substrate relative to the activity without substrate.

Lipolase™	94%
A.sporophora-variabilis lipolytic enzyme	39%

The results show that whereas Lipolase tends to remain totally in the aqueous phase under the conditions employed, the lipolytic enzyme from *A. sporophora-variabilis* has a higher affinity for olive oil, leaving less than 50% of the added activity in the aqueous phase after overnight incubation.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: Enzymatic Detergent Composition

(iii) NUMBER OF SEQUENCES: 19

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: DK 1236/94
(B) FILING DATE: 26-OCT-1994

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: DK 0828/95
(B) FILING DATE: 14-JUL-1995

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana
(B) STRAIN: NN000987 (ATCC 20430)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ser Ser Xaa Lys Gln Asx Tyr Arg Thr Ala Ser Glu Thr Glu Ile Gln
1 5 10 15
Ala His Thr

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana
(B) STRAIN: NN000591 (ATCC 20431)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ser Ser Xaa Xaa Gln Asx Tyr Arg Thr Ala Ser Glu Thr Glu Ile Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana
(B) STRAIN: NN000591 (ATCC 20431)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asx Tyr Arg Thr Ala Ser Glu Thr Glu Ile Gln Ala His Thr Phe Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ser Ser Xaa Lys Gln Asx Tyr Arg Thr Ala Ser Glu Thr Glu Ile Gln
1 5 10 15
Ala His Thr Phe Tyr
20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana
(B) STRAIN: NN000591 (ATCC 20431)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ile Ala Asn Ile Val Phe Val Pro Val Asx Tyr Pro Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana
(B) STRAIN: NN000591 (ATCC 20431)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Gly Phe Leu Asx Ser Tyr Asx Glu Val Gln Asx Gln Leu Val Ala Glu
1 5 10 15
Val Lys

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana
(B) STRAIN: NN000591 (ATCC 20431)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ile Val Val Ala Gly His Ser Leu Gly Gly Ala Thr Ala Val Leu Xaa
1 5 10 15
Ala Leu

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana
(B) STRAIN: NN000591 (ATCC 20431)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Pro Tyr Gln Arg Leu Val Asn Glu Arg Asp Ile Val Pro His Leu
1 5 10 15
Pro Pro Gly Ala Phe Gly Phe Leu Xaa Ala Gly
20 25

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana
(B) STRAIN: NN000591 (ATCC 20431)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Asp Ser Ser Leu Arg Val Cys Pro Asn Gly Ile Glu Thr Asp Asp Cys
1 5 10 15
Ser Asn Ser Ile Val Pro Phe
20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia blakesleeana
(B) STRAIN: NN000591 (ATCC 20431)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Thr Ser Val Ile Asp His
1 5

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia corymbifera
(B) STRAIN: NN100062 (IFO 8084)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Ser Thr Gln Asp Tyr Arg Ile Ala Ser Glu Ala Glu Ile Lys Ala His
1 5 10 15
Thr Phe Tyr Thr Ala Leu Ser Ala Asn
20 25

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia corymbifera
(B) STRAIN: NN100062 (IFO 8084)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Thr Val Ile Pro Gly Gly Gln Trp Ser Cys Pro His Cys Asp Val Ala
1 5 10 15
Pro Asn Leu Asn Ile Thr Lys
20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia corymbifera
(B) STRAIN: NN100062 (IFO 8084)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Gly Phe Leu Asp Ser Tyr Asn Glu Val Gln Asp Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia corymbifera
(B) STRAIN: NN100062 (IFO 8084)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Ala Gln Leu Asp Arg His Pro Gly Tyr Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia corymbifera
(B) STRAIN: NN100062 (IFO 8084)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Ile Val Val Thr Gly His Ser Leu Gly Gly Ala Thr Ala Val Leu Ser
1 5 10 15
Ala Leu Asp Leu Tyr His His Gly His Asp Asn Ile Glu Ile Tyr Thr
20 25 30
Gln Gly Gln Pro Arg Ile
35

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia corymbifera
(B) STRAIN: NN100062 (IFO 8084)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Ala Leu Asp Leu Tyr His His Gly His Asp Asn Ile Glu Ile Tyr Thr
1 5 10 15
Gln Gly Gln Pro Arg Ile Gly Gly Pro Glu Phe Ala Asn Tyr Val
20 25 30

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia corymbifera
(B) STRAIN: NN100062 (IFO 8084)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Ile Val Val Thr Gly His Ser Leu Gly Gly Ala Thr Ala Val Leu Ser
1 5 10 15

Ala Leu Asp Leu Tyr His His Gly His Asp Asn Ile Glu Ile Tyr Thr
20 25 30
Gln Gly Gln Pro Arg Ile Gly Gly Pro Glu Phe Ala Asn Tyr Val
35 40 45

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Absidia corymbifera
(B) STRAIN: NN100062 (IFO 8084)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Ile Pro Tyr Gln Arg Leu Val Asn Glu Arg Asp Ile Val Pro His Leu
1 5 10 15
Pro Pro Gly Ala Phe Gly Phe Leu His Ala Gly Glu Glu Phe Trp Ile
20 25 30
Met Lys

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Absidia corymbifera

(B) STRAIN: NN100062 (IFO 8084)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asp Ser Ser Leu Arg Val Cys Pro Asn Gly Ile Glu Thr Asp Asn Cys
1 5 10 15

Ser Asn Ser Ile Val Pro Phe
20

CLAIMS

1. An enzymatic detergent composition comprising a surfactant and an alkaline *Absidia* lipolytic enzyme.

2. The detergent composition of claim 1 wherein the lipolytic enzyme has a higher lipolytic enzyme activity at pH 9 than at pH 8 in the absence of free Ca^{++}.

3. The detergent composition of claim 1 or 2 wherein the lipolytic enzyme is derived from a strain of *Absidia* subgenus *Mycocladus* and has a lipolytic enzyme activity ratio of at least 0.15 OPID (pH 9 without free Ca^{++}) per LU.

4. The detergent composition of claim 3 wherein the strain belongs to *A. blakesleeana*.

5. The detergent composition of claim 3 wherein the strain belongs to *A. blakesleeana var. atrospora*.

6. The detergent composition of claim 3 wherein the strain belongs to *A. corymbifera*.

7. The detergent composition of claim 1 or 2 wherein the lipolytic enzyme is derived from a strain of *Absidia* Subgenus *Absidia* Group B.

8. The detergent composition of claim 7 wherein the strain belongs to *A. cylindrospora var. rhizomorpha* or *A. pseudocylindrospora*.

9. The detergent composition of claim 1 or 2 wherein the lipolytic enzyme is derived from a strain of *A. reflexa* and has a higher lipolytic enzyme activity at pH 10 than pH 9 in the absence of free Ca^{++}.

10. The detergent composition of claim 1 or 2 wherein the lipolytic enzyme is derived from a strain of *A. sporophora-variabilis* and retains more than 90% residual activity after 30 minutes incubation at pH 10, 45°C.

11. The detergent composition of claim 1 wherein the lipolytic enzyme contains an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 5, 6, 7, 8, 9 and 10.

12. The detergent composition of the claim 11 wherein the lipolytic enzyme contains two or more of said sequences and preferably contains all of said sequences.

13. The detergent composition of claim 1 wherein the lipolytic enzyme contains an amino acid sequence selected from the group consisting of SEQ ID NO: 11, 12, 13, 14, 17, 18 and 19.

14. The detergent composition of claim 13 wherein the lipolytic enzyme contains two or more of said sequences and preferably contains all of said sequences.

15. The detergent composition of any of claims 1-14 which further comprises 5-40% by weight of a detergent builder and which has a pH of 8-10.5 measured in an aqueous solution.

16. A method for removing fatty soiling from textile, comprising washing the textile in an aqueous solution comprising the detergent composition of any of claims 1-15.

17. The method of claim 16 wherein the aqueous solution comprises essentially no free Ca^{++} ions or contains free Ca^{++} ions in an amount below 1 mM, preferably below 0.2 mM.

18. An enzymatic detergent additive in the form of a non-dusting granulate, a stabilized liquid, a slurry, or a protected enzyme, which contains an *Absidia* lipolytic enzyme as an active component.

19. The enzymatic detergent additive of claim 18 wherein the lipolytic enzyme has a higher lipolytic enzyme activity at pH 9 than pH 8 in the absence of free Ca^{++}.

20. The enzymatic detergent additive of claim 18 or 19 wherein the lipolytic enzyme is derived from a strain belonging to *Absidia* subgenus *Mycocladus* and has a lipolytic enzyme activity ratio of at least 0.15 OPID (pH 9 without free Ca^{++}) per LU.

21. The enzymatic detergent additive of claim 20 wherein the strain belongs to *A. blakesleeana*.

22. The enzymatic detergent additive of claim 20 wherein the strain belongs to *A. blakesleeana var. atrospora*.

23. The enzymatic detergent additive of claim 20 wherein the strain belongs to *A. corymbifera*.

24. The enzymatic detergent additive of claim 18 or 19 wherein the lipolytic enzyme is derived from a strain of *Absidia* Subgenus *Absidia* Group B.

25. The enzymatic detergent additive of claim 24 wherein the strain belongs to *A. cylindrospora var. rhizomorpha* or *A. pseudocylindrospora*.

26. The enzymatic detergent additive of claim 18 or 19 wherein the lipolytic enzyme is derived from a strain of *A. reflexa* and has a higher lipolytic enzyme activity at pH 10 than pH 9 in the absence of free Ca^{++}.

27. The enzymatic detergent additive of claim 18 or 19 wherein the lipolytic enzyme is derived from a strain of *A. sporophora-variabilis* and retains more than 90% residual activity after 30 minutes incubation at pH 10, 45°C.

28. The enzymatic detergent additive of claim 18 wherein the lipolytic enzyme contains an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 5, 6, 7, 8, 9 and 10.

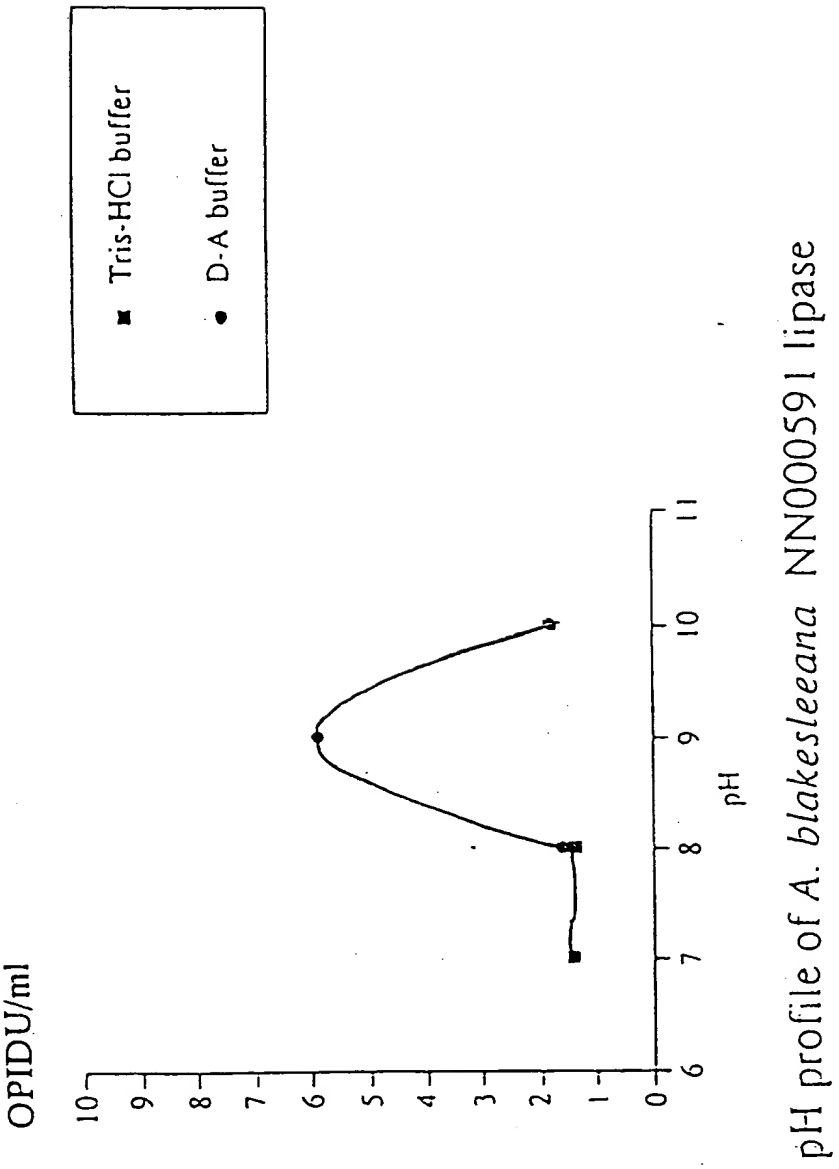
29. The enzymatic detergent additive of claim 28 wherein the lipolytic enzyme contains two or more of said sequences and preferably contains all of said sequences.

30. The enzymatic detergent additive of claim 18 wherein the lipolytic enzyme contains an amino acid sequence selected from the group consisting of SEQ ID NO: 11, 12, 13, 14, 17, 18 and 19.

31. The enzymatic detergent additive of claim 30 wherein the lipolytic enzyme contains two or more of said sequences and preferably contains all of said sequences.

32. A lipolytic enzyme which is derived from a strain of *A. sporophora-variabilis* and retains more than 90% residual activity after 30 minutes incubation at pH 10, 45°C.

33. A lipolytic enzyme which is derived from a strain of *A. reflexa* and has a higher lipolytic enzyme activity at pH 10 than at pH 9 in the absence of Ca^{++}.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D-A buffer
OPIDU/ml
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9
8
7
6
5
4
3
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1
0
6
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8
9
10
11
pH
pH profile of A. blakesleeana NN000591 lipase

FIG. 1

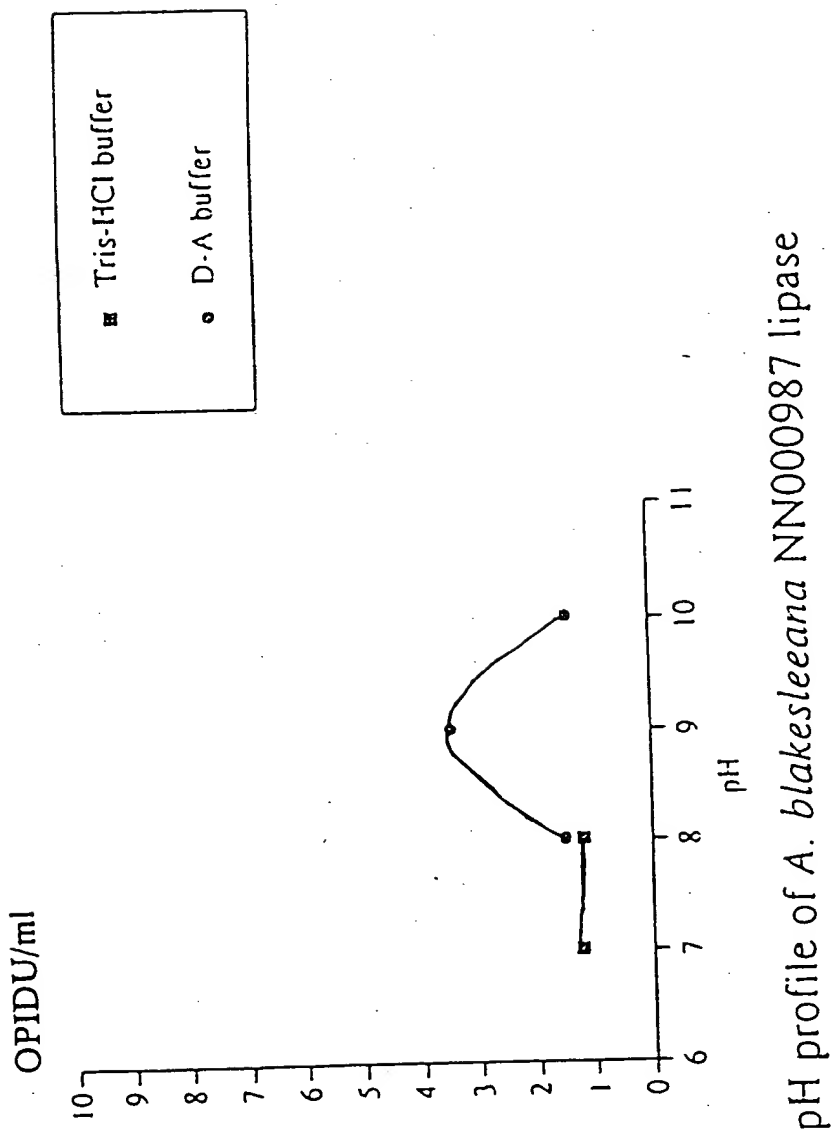
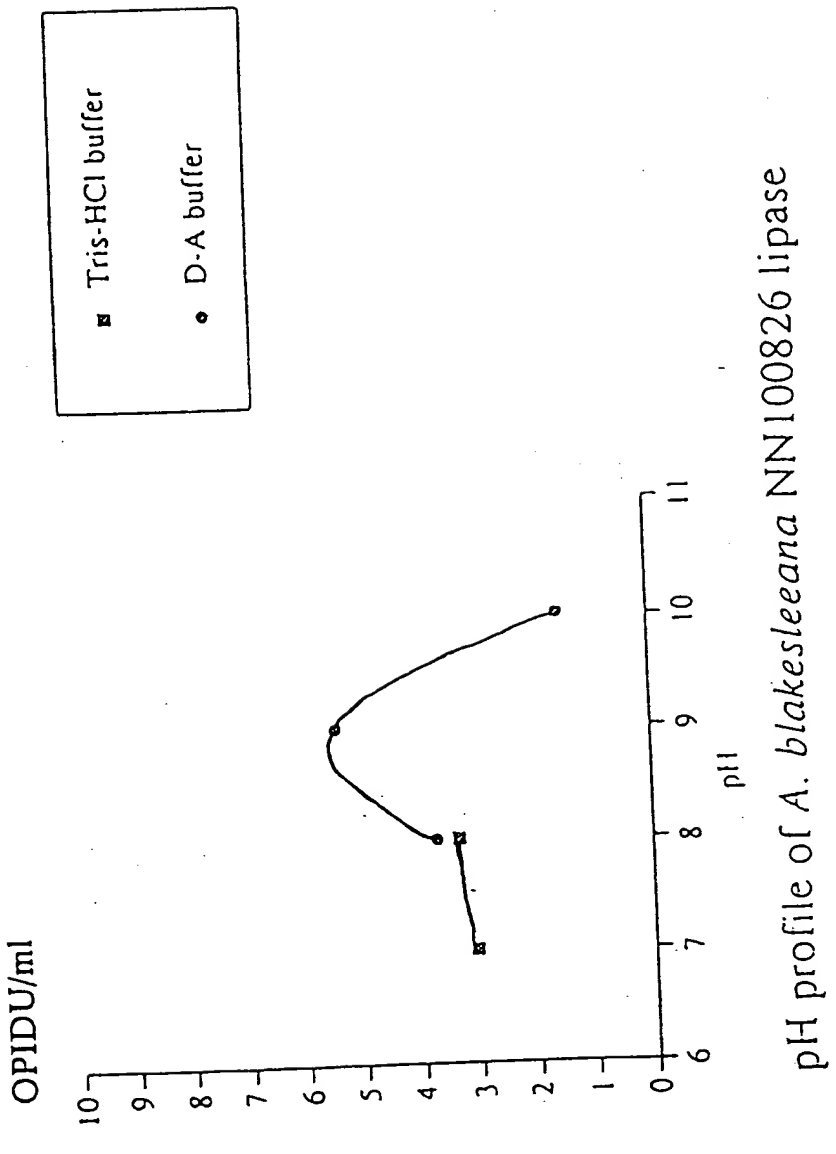


FIG. 2

SUBSTITUTE SHEET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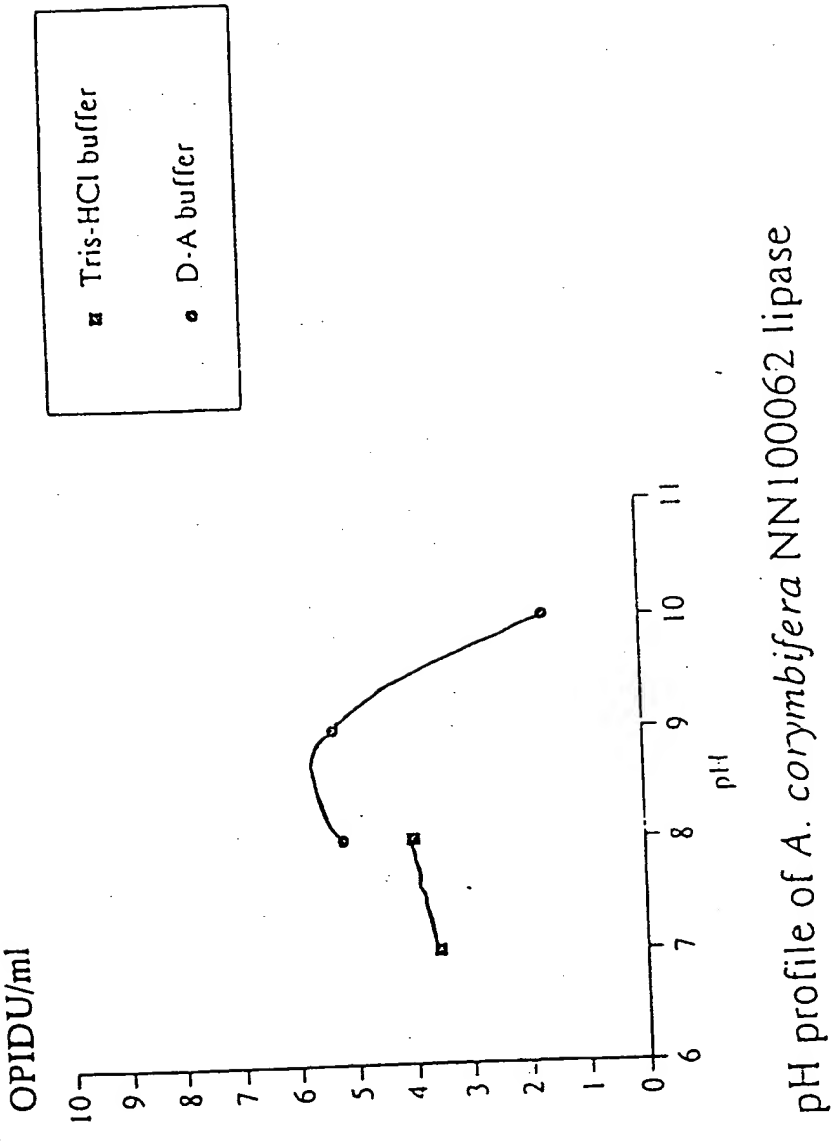
D-A buffer
OPIDU/ml
10
9
8
7
6
5
4
3
2
1
0
6
7
8
9
10
11
pH
pH profile of A. blakesleeana NN100826 lipase

FIG. 3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D-A buffer
OPIDU/ml
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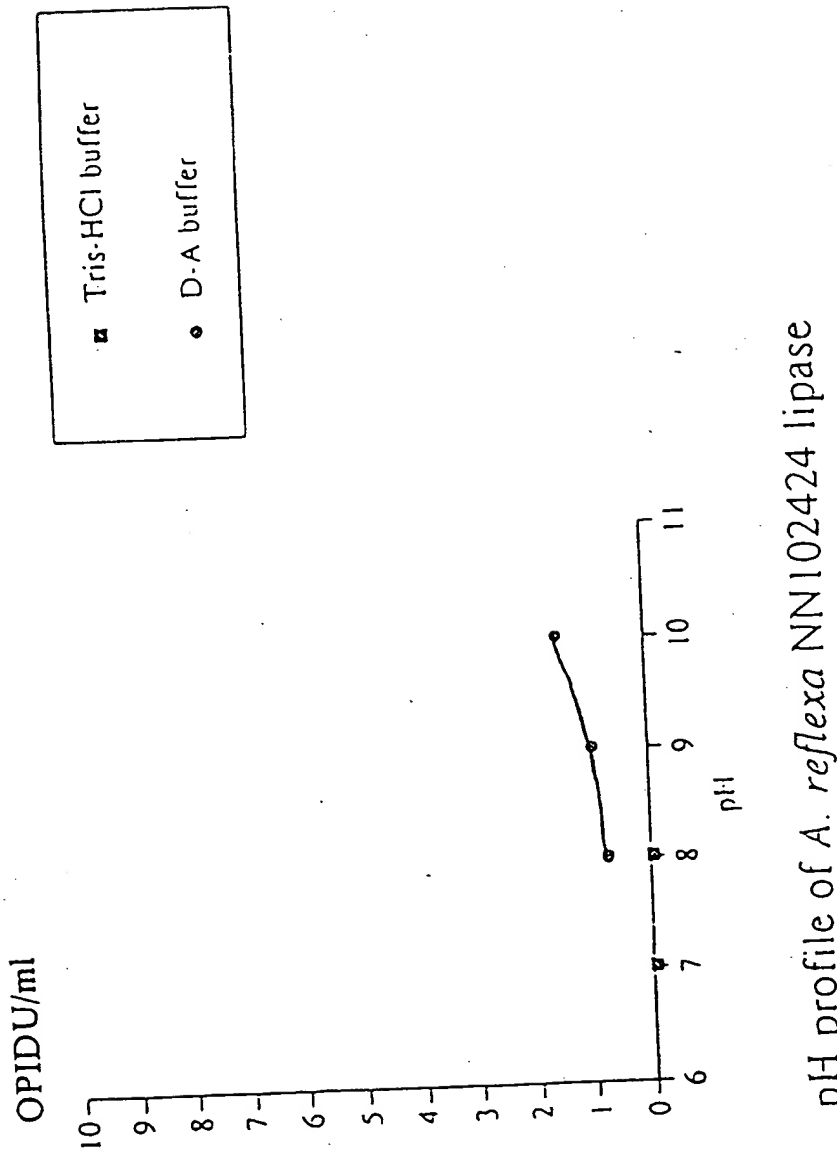
pH
pH profile of A. corymbifera NN100062 lipase

FIG. 4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D-A buffer
OPIDU/ml
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9
8
7
6
5
4
3
2
1
0
6
7
8
9
10
11
pH
pH profile of A. reflexa NN102424 lipase

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00424

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/20, C11D 3/386
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N, C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, BIOSIS, EMBL, GENBANK, DDBJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9414940 A1 (NOVO NORDISK A/S), 7 July 1994 (07.07.94), see claim 1, abstract	1-10,15-27, 32-33
Y		11-14,28-31
	--	
X	WO 8700859 A1 (GISTBROCADES N.V.), 12 February 1987 (12.02.87), see abstract, claim 1	1-10,15-27, 32-33
Y		11-14,28-31
	--	
X	EP 0385401 A1 (OCCIDENTAL CHEMICAL CORPORATION), 5 Sept 1990 (05.09.90), abstract	1-10,15-27, 32-33
Y		11-14,28-31
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"B" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 February 1996	21.02.1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Yvonne Siösteen Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 95/00424

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 9401541 A1 (NOVO NORDISK A/S), 20 January 1994 (20.01.94), see sequence disting	11-14,28-31
Y	EMBL, Genbank, DDBJ, accession no. A34959, Boel, E. et al: "Rhizomucor miehei triglyceride lipase is synthesized as a precursor"; & Lipids 23, 701-706, 1988	11-14,28-31
Y	EP 0238023 A2 (NOVO INDUSTRI A/S), 23 Sept 1987 (23.09.87), see claims 9,10,19,21	11-14,28-31
Y	EP 0489718 A1 (NOVO NORDISK A/S), 10 June 1992 (10.06.92), see claims 2,4	11-14,28-31
A	US 3634195 A (MILES LABORATORIES, INC. ELKHART, IND.), 11 January 1972 (11.01.72)	1-33
A	Chemical Abstracts, Volume 95, No 13, 28 Sept 1981 (28.09.81), (Columbus, Ohio, USA), Satyanarayana, T et al, "Lipolytic activity of thermophilic fungi of paddy straw compost", page 344, THE ABSTRACT No 111511y, Curr. Sci. 1981, 50 (15), 680-682	1-33
A	Dialog Information Services, file 351, Derwent WPI, Dialog accession no. 008384112, WPI accession no. 90-271113/36, TANABE SEIYAKU KK et al: "Optically active propionic acid ester(s) prodn. - by hydrolysis of opt. isomer of racemic propionic acid ester(s)using asymmetrically; HYDROLYSIS BIO CATALYST"; JP,A,2190195, 900726, 9036 (Basic)	1-33

INTERNATIONAL SEARCH REPORT
Information on patent family members

05/02/96

International application No.
PCT/DK 95/00424

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